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 NEWS  8  OCT 28   KOREAPAT now available on STN
 NEWS  9  NOV 18   Current-awareness alerts, saved answer sets, and current
                   search transcripts to be affected by CERAB, COMPUAB, ELCOM,
                   and SOLIDSTATE reloads
 NEWS 10  NOV 30   PHAR reloaded with additional data
 NEWS 11  DEC 01   LISA now available on STN

 NEWS EXPRESS  OCTOBER 29 CURRENT WINDOWS VERSION IS V7.01A, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2004
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FILE 'HOME' ENTERED AT 18:41:50 ON 07 DEC 2004

=> file medline, biosis, biotechds, embase, wpids, dgene, uspatful, hcaplus
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 18:42:21 ON 07 DEC 2004

FILE 'BIOSIS' ENTERED AT 18:42:21 ON 07 DEC 2004
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FILE 'BIOTECHDS' ENTERED AT 18:42:21 ON 07 DEC 2004
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FILE 'EMBASE' ENTERED AT 18:42:21 ON 07 DEC 2004

FILE 'WPIDS' ENTERED AT 18:42:21 ON 07 DEC 2004

FILE 'DGENE' ENTERED AT 18:42:21 ON 07 DEC 2004

FILE 'USPATFULL' ENTERED AT 18:42:21 ON 07 DEC 2004

FILE 'HCAPLUS' ENTERED AT 18:42:21 ON 07 DEC 2004


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=> s albumin and fusion protein
   7 FILES SEARCHED...
L1        28181 ALBUMIN AND FUSION PROTEIN

=> s l1 and intereferon
L2            5 L1 AND INTEREFERON

=> d l2 ti abs ibib tot

L2   ANSWER 1 OF 5  USPATFULL on STN
TI     123 human secreted proteins
AB     The present invention relates to novel human secreted proteins and
       isolated nucleic acids containing the coding regions of the genes
       encoding such proteins. Also provided are vectors, host cells,
       antibodies, and recombinant methods for producing human secreted
       proteins. The invention further relates to diagnostic and therapeutic
       methods useful for diagnosing and treating diseases, disorders, and/or
       conditions related to these novel human secreted proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER:        2004:58184  USPATFULL
TITLE:                   123 human secreted proteins
INVENTOR(S):             Fischer, Carrie L., Burke, VA, UNITED STATES
                         Rosen, Craig A., Laytonsville, MD, UNITED STATES
                         Soppet, Daniel R., Centreville, VA, UNITED STATES
                         Ruben, Steven M., Olney, MD, UNITED STATES
                         Kyaw, Hla, Frederick, MD, UNITED STATES
                         Li, Yi, Sunnyvale, CA, UNITED STATES
                         Zeng, Zhizhen, Lansdale, PA, UNITED STATES
                         LaFleur, David W., Washington, DC, UNITED STATES
                         Moore, Paul A., Germantown, MD, UNITED STATES
                         Shi, Yanggu, Gaithersburg, MD, UNITED STATES
                         Olsen, Henrik, Gaithersburg, MD, UNITED STATES
                         Ebner, Reinhard, Gaithersburg, MD, UNITED STATES
                         Birse, Charles E., North Potomac, MD, UNITED STATES

                              NUMBER         KIND    DATE
                         ---------- ------ --------
PATENT INFORMATION:      US 2004044191      A1   20040304
APPLICATION INFO.:       US 2001-973278     A1   20011010  (9)
RELATED APPLN. INFO.:    Continuation-in-part of Ser. No. US 1999-227357, filed
                         on 8 Jan 1999, GRANTED, Pat. No. US 6342581
                         Continuation-in-part of Ser. No. WO 1998-US13684, filed
                         on 7 Jul 1998, UNKNOWN
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	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-239899P	20001013 (60)
	US 1997-51926P	19970708 (60)
	US 1997-52793P	19970708 (60)
	US 1997-51925P	19970708 (60)
	US 1997-51929P	19970708 (60)
	US 1997-52803P	19970708 (60)
	US 1997-52732P	19970708 (60)
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	US 1997-51932P	19970708 (60)
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	US 1997-55954P	19970818 (60)
	US 1997-58785P	19970912 (60)
	US 1997-58664P	19970912 (60)
	US 1997-58660P	19970912 (60)
	US 1997-58661P	19970912 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Page(s)
LINE COUNT: 36492
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 2 OF 5 USPATFULL on STN
TI 123 human secreted proteins
AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:93053 USPATFULL
TITLE: 123 human secreted proteins
INVENTOR(S): Fischer, Carrie L., Burke, VA, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Soppet, Daniel R., Centreville, VA, UNITED STATES
Ruben, Steven M., Olney, MD, UNITED STATES
Kyaw, Hla, Frederick, MD, UNITED STATES
Li, Yi, Sunnyvale, CA, UNITED STATES

Zeng, Zhizhen, Lansdale, PA, UNITED STATES
LaFleur, David W., Washington, DC, UNITED STATES
Moore, Paul A., Germantown, MD, UNITED STATES
Shi, Yanggu, Gaithersburg, MD, UNITED STATES
Olsen, Henrik S., Gaithersburg, MD, UNITED STATES
Ebner, Reinhard, Gaithersburg, MD, UNITED STATES
Brewer, Laurie A., St. Paul, MN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003064412	A1	20030403
APPLICATION INFO.:	US 2001-984490	A1	20011030 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1999-227357, filed on 8 Jan 1999, PATENTED Continuation-in-part of Ser. No. WO 1998-US13684, filed on 7 Jul 1998, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-51926P	19970708 (60)
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	US 1997-51925P	19970708 (60)
	US 1997-51929P	19970708 (60)
	US 1997-52803P	19970708 (60)
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	US 1997-55954P	19970818 (60)
	US 1997-58785P	19970912 (60)
	US 1997-58664P	19970912 (60)
	US 1997-58660P	19970912 (60)
	US 1997-58661P	19970912 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 70
EXEMPLARY CLAIM: 1
LINE COUNT: 20255
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 3 OF 5 USPATFULL on STN
TI Secreted protein HLHFP03
AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells,

antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2003:30238 USPATFULL

TITLE: Secreted protein HLHFP03

INVENTOR(S): Fischer, Carrie L., Burke, VA, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Soppet, Daniel R., Centreville, VA, UNITED STATES
Ruben, Steven M., Olney, MD, UNITED STATES
Kyaw, Hla, Frederick, MD, UNITED STATES
Li, Yi, Sunnyvale, CA, UNITED STATES
Zeng, Zhizhen, Lansdale, PA, UNITED STATES
LaFleur, David W., Washington, DC, UNITED STATES
Moore, Paul A., Germantown, MD, UNITED STATES
Shi, Yanggu, Gaithersburg, MD, UNITED STATES
Olsen, Henrik S., Gaithersburg, MD, UNITED STATES
Ebner, Reinhard, Gaithersburg, MD, UNITED STATES
Brewer, Laurie A., St. Paul, MN, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003022185	A1	20030130
APPLICATION INFO.:	US 2001-983802	A1	20011025 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-227357, filed on 8 Jan 1999, GRANTED, Pat. No. US 6342581 Continuation-in-part of Ser. No. WO 1998-US13684, filed on 7 Jul 1998, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-51926P	19970708 (60)
	US 1997-52793P	19970708 (60)
	US 1997-51925P	19970708 (60)
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	US 1997-58785P	19970912 (60)
	US 1997-58664P	19970912 (60)
	US 1997-58660P	19970912 (60)

	US 1997-58661P 19970912 (60)
DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS:	24
EXEMPLARY CLAIM:	1
LINE COUNT:	19390

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 4 OF 5 USPATFULL on STN

TI Secreted protein HLHFP03

AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:19393 USPATFULL
TITLE:	Secreted protein HLHFP03
INVENTOR(S):	Rosen, Craig A., Laytonsville, MD, United States Ruben, Steven M., Olney, MD, United States Olsen, Henrik S., Gaithersburg, MD, United States Ebner, Reinhard, Gaithersburg, MD, United States
PATENT ASSIGNEE(S):	Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6342581	B1	20020129
APPLICATION INFO.:	US 1999-227357		19990108 (9)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. WO 1998-US13684, filed on 7 Jul 1998		

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-58785P	19970912 (60)
	US 1997-58664P	19970912 (60)
	US 1997-58660P	19970912 (60)
	US 1997-58661P	19970912 (60)
	US 1997-55722P	19970818 (60)
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	US 1997-52793P	19970708 (60)
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	US 1997-51929P	19970708 (60)
	US 1997-52803P	19970708 (60)
	US 1997-52732P	19970708 (60)
	US 1997-51931P	19970708 (60)
	US 1997-51932P	19970708 (60)
	US 1997-51916P	19970708 (60)

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                        US 1997-51930P     19970708 (60)
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                        US 1997-52795P     19970708 (60)
                        US 1997-51919P     19970708 (60)
                        US 1997-51928P     19970708 (60)
DOCUMENT TYPE:          Utility
FILE SEGMENT:           GRANTED
PRIMARY EXAMINER:       Myers, Carla J.
ASSISTANT EXAMINER:     Spiegler, Alexander H.
LEGAL REPRESENTATIVE:   Human Genome Sciences, Inc.
NUMBER OF CLAIMS:       46
EXEMPLARY CLAIM:        1
NUMBER OF DRAWINGS:     0 Drawing Figure(s); 0 Drawing Page(s)
LINE COUNT:             18742
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2   ANSWER 5 OF 5  USPATFULL on STN
TI     Recombinant chimeric virus and uses thereof
AB     This invention provides a recombinant herpesvirus of turkeys-Marek's
       disease virus chimera comprising a herpesvirus of turkeys unique long
       viral genome region and a Marek's disease virus unique short viral
       genome region.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER:       2001:18001  USPATFULL
TITLE:                  Recombinant chimeric virus and uses thereof
INVENTOR(S):            Cochran, Mark D., Carlsbad, CA, United States
                        Wild, Martha A., San Diego, CA, United States
                        Winslow, Barbara J., Delmar, CA, United States
PATENT ASSIGNEE(S):     Schering-Plough Veterinary Corp., Reno, NV, United
                        States (U.S. corporation)

                             NUMBER       KIND    DATE
                        ---------------- ------ --------
PATENT INFORMATION:     US 6183753         B1   20010206
APPLICATION INFO.:      US 1997-804372          19970221  (8)
RELATED APPLN. INFO.:   Continuation-in-part of Ser. No. US 1996-663566, filed
                        on 13 Jun 1996, now patented, Pat. No. US 5853733
                        Continuation-in-part of Ser. No. WO 1995-US10245, filed
                        on 9 Aug 1995 Continuation-in-part of Ser. No. US
                        1994-288065, filed on 9 Aug 1994, now patented, Pat.
                        No. US 5961982
DOCUMENT TYPE:          Utility
FILE SEGMENT:           Granted
PRIMARY EXAMINER:       Salimi, Ali
NUMBER OF CLAIMS:       20
EXEMPLARY CLAIM:        1
NUMBER OF DRAWINGS:     6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT:             3184
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> d his

     (FILE 'HOME' ENTERED AT 18:41:50 ON 07 DEC 2004)

     FILE 'MEDLINE, BIOSIS, BIOTECHDS, EMBASE, WPIDS, DGENE, USPATFULL,
     HCAPLUS' ENTERED AT 18:42:21 ON 07 DEC 2004
L1        28181 S ALBUMIN AND FUSION PROTEIN
L2            5 S L1 AND INTEREFERON

=> s interferon alpha or beta
```

L3 3732316 INTERFERON ALPHA OR BETA

=> s l3 and fusion
L4 119770 L3 AND FUSION

=> s l4 and albumin
L5 25714 L4 AND ALBUMIN

=> s l5 and HSA
L6 1525 L5 AND HSA

=> d l6 ti abs ibib 1-20

L6 ANSWER 1 OF 1525 MEDLINE on STN

TI An IFN-**beta**-**albumin** **fusion** protein that displays improved pharmacokinetic and pharmacodynamic properties in nonhuman primates.

AB The long half-life and stability of human serum **albumin** (**HSA**) make it an attractive candidate for **fusion** to short-lived therapeutic proteins. Albuferon (Human Genome Sciences [HGS], Inc., Rockville, MD) **beta** is a novel recombinant protein derived from a gene **fusion** of interferon-**beta** (IFN-**beta**) and **HSA**. In vitro, Albuferon **beta** displays antiviral and antiproliferative activities and triggers the IFN-stimulated response element (ISRE) signal transduction pathway. Array analysis of 5694 independent genes in Daudi-treated cells revealed that Albuferon **beta** and IFN-**beta** induce the expression of an identical set of 30 genes, including 9 previously not identified. In rhesus monkeys administered a dose of 50 microg/kg intravenously (i.v.) or subcutaneously (s.c.) or 300 microg/kg s.c., Albuferon **beta** demonstrated favorable pharmacokinetic properties. Subcutaneous bioavailability was 87%, plasma clearance at 4.7-5.7 ml/h/kg was approximately 140-fold lower than that of IFN-**beta**, and the terminal half-life was 36-40 h compared with 8 h for IFN-**beta**. Importantly, Albuferon **beta** induced sustained increases in serum neopterin levels and 2',5' mRNA expression. At a molar dose equivalent to one-half the dose of IFN-**beta**, Albuferon **beta** elicited comparable neopterin responses and significantly higher 2',5'-OAS mRNA levels in rhesus monkeys. The enhanced in vivo pharmacologic properties of IFN-**beta** when fused to serum **albumin** suggest a clinical opportunity for improved IFN-**beta** therapy.

ACCESSION NUMBER: 2003128795 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12639296
TITLE: An IFN-**beta**-**albumin** **fusion** protein that displays improved pharmacokinetic and pharmacodynamic properties in nonhuman primates.
AUTHOR: Sung Cynthia; Nardelli Bernardetta; LaFleur David W; Blatter Erich; Corcoran Marta; Olsen Henrik S; Birse Charles E; Pickeral Oxana K; Zhang Junli; Shah Devanshi; Moody Gordon; Gentz Solange; Beebe Lisa; Moore Paul A
CORPORATE SOURCE: Human Genome Sciences, Inc, Rockville, MD 20850, USA.
SOURCE: Journal of interferon & cytokine research : official journal of the International Society for Interferon and Cytokine Research, (2003 Jan) 23 (1) 25-36. Journal code: 9507088. ISSN: 1079-9907.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 20030320
Last Updated on STN: 20030928
Entered Medline: 20030926

L6 ANSWER 2 OF 1525 MEDLINE on STN
TI Expression of recombinant psoriasis-associated fatty acid binding protein in Escherichia coli: gel electrophoretic characterization, analysis of binding properties and comparison with human serum **albumin**.
AB The psoriasis-associated fatty acid binding protein (PA-FABP, also known as FABP5) is a novel keratinocyte protein that is highly up-regulated in psoriatic plaques (P. Madsen, H. H. Rasmussen, H. Leffers, B. Honore and J. E. Celis, J. Invest. Dermatol. 1992, 99, 299-305). Here we have expressed PA-FABP in Escherichia coli as a **fusion** protein containing an NH2-terminal hexa-His tag followed by a factor Xa cleavage site. The recombinant protein was expressed at a level of about 30% of the soluble proteins and was purified to homogeneity using a simple two-step protocol consisting of affinity chromatography on Ni2+-nitrilotriacetic acid agarose followed by gel filtration. The recombinant protein was then digested with factor Xa and characterized by two-dimensional gel electrophoresis. The ability of PA-FABP to bind saturated fatty acids ranging from 6 to 16 carbons was determined directly by dialysis and compared to human serum **albumin** (**HSA**).
The results showed that PA-FABP binds multiple molecules of the fatty acids hexanoate (C6:0), octanoate (C8:0), decanoate (C10:0) and laurate (C12:0), all with a K1 of about 10(4) M(-1), and myristate (C14:0) with a K1 of 4.4 X 10(5) M(-1). Palmitate (C16:0) also bound strongly with multiple molecules. Due to the very low solubility of palmitate its affinity to PA-FABP was measured relatively to **HSA** and found to be 8.1 times lower. At ligand/protein ratios below 1, all fatty acids bound to PA-FABP with about one to three orders of magnitude lower affinity than to **HSA**. The difference in the fatty acid binding properties of the two proteins may reflect differences in their three-dimensional structures, which in the case of PA-FABP consists mainly of **beta**-sheets while **HSA** contains predominantly alpha-helices.

ACCESSION NUMBER: 1998384066 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9719561
TITLE: Expression of recombinant psoriasis-associated fatty acid binding protein in Escherichia coli: gel electrophoretic characterization, analysis of binding properties and comparison with human serum **albumin**.
AUTHOR: Vorum H; Madsen P; Svendsen I; Cells J E; Honore B
CORPORATE SOURCE: Department of Medical Biochemistry and Danish Centre for Human Genome Research, University of Aarhus.
SOURCE: Electrophoresis, (1998 Jul) 19 (10) 1793-802.
Journal code: 8204476. ISSN: 0173-0835.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981109

L6 ANSWER 3 OF 1525 MEDLINE on STN
TI Inhibition of influenza virus **fusion** by polyanionic proteins.
AB Anionic charge-modified human serum **albumin** (**HSA**) has previously been shown to exert potent in vitro activity against human immunodeficiency virus type 1 (HIV-1). In these studies, introduction of the additional negative charges was performed by derivatizing the epsilon-amino groups of lysine residues with succinic (Suc-**HSA**) or cis-aconitic anhydride (Aco-**HSA**), by which primary amino groups are replaced with carboxylic acids. The anti-HIV-1 activity was related to inhibition of gp41-mediated membrane **fusion**. Here, we investigated the activity of aconitylated and succinylated proteins on influenza virus membrane **fusion**, which is mediated by the viral membrane glycoprotein hemagglutinin (HA). Aco-**HSA** and Suc-

HSA markedly inhibited the rates and extents of **fusion** of fluorescently labeled virosomes bearing influenza HA, with target membranes derived from erythrocytes. The inhibitory activity was dependent on the overall negative-charge density; **HSA** modified with 36 or less extra negative charges failed to inhibit **fusion**. The inhibition of **fusion** showed a certain degree of specificity for the protein carrying the negative charges: polyanionic **HSA** and **beta**-lactoglobulin A derivatives had **fusion** -inhibitory activity, whereas succinylated BSA, lactalbumin, lactoferrin, lysozyme, and transferrin were inactive. Aco60-**HSA** and Aco-**beta**-lactoglobulin A inhibited influenza virus membrane **fusion** in a concentration-dependent manner, IC50 values being about 4 and 10 microg/mL, respectively. HA-mediated membrane **fusion** is pH dependent. Aco60-**HSA** did not induce a shift in the pH threshold or in the pH optimum. **Fusion** with liposomes of another low pH-dependent virus, Semliki Forest virus, was not specifically affected by any of the compounds reported here. In view of some structural and functional similarities between influenza HA and the HIV-1 gp120/gp41 complex, it is tempting to postulate that the current results might have some implications for the anti-HIV-1 mechanism of polyanionic proteins.

ACCESSION NUMBER: 97316839 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9174113
TITLE: Inhibition of influenza virus **fusion** by polyanionic proteins.
AUTHOR: Schoen P; Corver J; Meijer D K; Wilschut J; Swart P J
CORPORATE SOURCE: Groningen Utrecht Institute for Drug Exploration (GUIDE), University of Groningen, Department of Physiological Chemistry, Faculty of Medical Sciences, The Netherlands.. p.j.schoen@med.rug.nl
SOURCE: Biochemical pharmacology, (1997 Apr 4) 53 (7) 995-1003. Journal code: 0101032. ISSN: 0006-2952.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970630
Last Updated on STN: 19970630
Entered Medline: 19970617

L6 ANSWER 4 OF 1525 MEDLINE on STN
TI **Beta**-lactoglobulin/human serum **albumin fusion** genes do not respond accurately to signals from the extracellular matrix in mammary epithelial cells from transgenic mice.
AB Mammary epithelial cell cultures from transgenic mice carrying the human serum **albumin** (**HSA**) gene or minigenes behind the regulatory sequences of the ovine **beta**-lactoglobulin gene were analyzed. Previously, we demonstrated that non-**HSA**-secreting transgenic strains retain the potential to express the **HSA** transgene in vitro and that mammary epithelial cell cultures from non-**HSA**-secreting strains express higher levels of **HSA** when grown on tissue culture plastic than they do when grown on collagen. In this study we studied the expression of BLG/**HSA** **fusion** genes in epithelial cell cultures of additional transgenic strains and additional substrata. Our results show that: (1) The BLG/**HSA** **fusion** gene in only one of seven **HSA**-secreting or nonsecreting transgenic strains tested accurately responded to signals from the EHS matrix; (2) **HSA** DNA sequences dominantly affected the activity of BLG as well as the whey acidic protein promoters; and (3) HGF/SF induced both milk proteins and **HSA** gene expression. These results suggest that the response to the extra cellular matrix (ECM) plays a key role in the expression of BLG/**HSA** **fusion** genes and that the function of the regulatory elements within the promoter

regions of milk protein genes involved in response to the ECM, in developmental and in tissue specificity, very much depend on the downstream gene sequences.

ACCESSION NUMBER: 97048141 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8892982
TITLE: **Beta**-lactoglobulin/human serum **albumin** **fusion** genes do not respond accurately to signals from the extracellular matrix in mammary epithelial cells from transgenic mice.
AUTHOR: Ilan N; Barash I; Raikhinstein M; Faerman A; Shani M
CORPORATE SOURCE: Institute of Animal Science, The Volcani Center, Bet Dagan, Israel.
SOURCE: Experimental cell research, (1996 Oct 10) 228 (1) 146-59. Journal code: 0373226. ISSN: 0014-4827.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961224

L6 ANSWER 5 OF 1525 MEDLINE on STN
TI [Obtaining and properties of recombinant protein G]. Poluchenie i svoistva rekombinantnogo belka G.
AB A gene for G protein from Streptococcus strain G148 was cloned in Escherichia coli, which gave rise to several plasmids. One plasmid containing a 1.5 kb insert coding for entire G protein with 63 kD. This protein had both an IgG binding capacity and **albumin**-binding activity. The second plasmid containing a 0.7 kD insert coded for protein with MM of 38 kD and had only an IgG-binding activity. The third coding for protein with 25 kD has only **albumin**-binding activity. After subcloning the 1.5-kb insert into the other vector pSP65 and analysing the nucleotide sequence of this insert both in pSP65 vector, the authors came to the conclusion that the proteins obtained are **fusion** protein of G protein and **beta**-galactosidase. All the proteins were prepared by affinity chromatography on IgG sepharose or on **HSA** sepharose. The interaction between G protein and polyclonal and monoclonal IgG of the reactions between G protein and human IgG have determined.

ACCESSION NUMBER: 96250973 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8672962
TITLE: [Obtaining and properties of recombinant protein G]. Poluchenie i svoistva rekombinantnogo belka G.
AUTHOR: Gupalova T V; Golubkov V I; Suvorov A N; Andreev A S; Dmitriev A V; Totolian A A
SOURCE: Vestnik Rossiiskoi akademii meditsinskikh nauk / Rossiiskaia akademiia meditsinskikh nauk, (1996) (3) 44-50. Journal code: 9215641. ISSN: 0869-6047.
PUB. COUNTRY: RUSSIA: Russian Federation
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960822
Last Updated on STN: 19960822
Entered Medline: 19960812

L6 ANSWER 6 OF 1525 MEDLINE on STN
TI Specific combinations of human serum **albumin** introns direct high level expression of **albumin** in transfected COS cells and in the milk of transgenic mice.
AB A new series of expression vectors, each comprised of the **beta**

-lactoglobulin (BLG) promoter driving one of a variety of human serum **albumin** (**HSA**) minigenes or the entire gene, were evaluated for their ability to direct expression of **HSA** in vitro in COS tissue culture cells and into the milk of transgenic mice. Vectors directed a hierarchy of expression levels in vitro, dependent upon the specific complement of **HSA** introns included. **HSA** introns acted in a synergistic manner. In addition, minigenes comprised of specific subsets of introns were more efficacious than the entire **HSA** gene with all of its introns. Transgenic mice expressed as much as 10 mg ml-1 of **HSA** in their milk. Vectors comprised of specific intron subsets directed levels at 1 mg ml-1 or greater in the milk of 20% of generated transgenics. A statistical correlation between the expression level trend in vitro with the trend of expression in vivo (% which express) at detectable levels ($p = 0.0015$) and at the level of greater than 0.1 mg ml-1 ($p = 0.0156$) was demonstrated. A weak correlation existed ($p = 0.0526$) at in vivo levels of 1 mg ml-1 or greater. These new vectors are expected to direct the production of high levels of **HSA** in the milk of a large percentage of generated transgenic dairy animals.

ACCESSION NUMBER: 95093481 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8000433
TITLE: Specific combinations of human serum **albumin** introns direct high level expression of **albumin** in transfected COS cells and in the milk of transgenic mice.
AUTHOR: Hurwitz D R; Nathan M; Barash I; Ilan N; Shani M
CORPORATE SOURCE: Rhone-Poulenc Rorer Central Research, Collegeville, PA 19426.
SOURCE: Transgenic research, (1994 Nov) 3 (6) 365-75. Journal code: 9209120. ISSN: 0962-8819.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19950215
Entered Medline: 19950120

L6 ANSWER 7 OF 1525 MEDLINE on STN
TI Ectopic expression of **beta**-lactoglobulin/human serum **albumin fusion** genes in transgenic mice: hormonal regulation and in situ localization.
AB We produced transgenic mice carrying the native sheep **beta** -lactoglobulin (BLG) or **fusion** genes composed of the BLG promoter and human serum **albumin** (**HSA**) minigenes. BLG was expressed exclusively in the mammary glands of the virgin and lactating transgenic mice evaluated. In contrast, transgenic females carrying the BLG/**HSA fusion** constructs also expressed the **HSA** RNA ectopically in skeletal muscle, kidney, brain, spleen, salivary gland and skin. Ectopic expression of **HSA** RNA was detected only in strains that express the transgene in the mammary gland. There was no obvious correlation between the level of the **HSA** RNA expressed in the mammary gland and that found ectopically. In three transgenic strains analysed, the expression of **HSA** RNA in kidney and skeletal muscle increased during pregnancy and lactation, whereas in the brain **HSA** expression decreased during lactation in one of the strains. **HSA** protein was synthesized in skeletal muscle and skin of strain #23 and its level was higher in lactating mice compared with virgin mice. Expression of **HSA** was also analysed in males and was found to be more stringently controlled than in females of the same strains. In situ hybridization analyses localized the expressed transgene in the skin, kidney, brain and salivary glands of various transgenic strains. Distinct strain-specific and cell-type

specific **HSA** expression patterns were observed in the skin. This is in contrast to the exclusive expression of the **HSA** transgene in epithelial cells surrounding the alveoli of the mammary gland. Taken together, these results suggest that the absence of sufficient mammary-specific regulatory elements in the BLG promoter sequences and/or the juxtaposition of the BLG promoter with the **HSA** coding sequences leads to novel tissue- and cell-specific expression in ectopic tissues of transgenic mice.

ACCESSION NUMBER: 94297617 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8025593
TITLE: Ectopic expression of **beta**-lactoglobulin/human serum **albumin fusion** genes in transgenic mice: hormonal regulation and in situ localization.
AUTHOR: Barash I; Faerman A; Ratovitsky T; Puzis R; Nathan M; Hurwitz D R; Shani M
CORPORATE SOURCE: Institute of Animal Science, Volcani Center, Bet Dagan, Israel.
SOURCE: Transgenic research, (1994 May) 3 (3) 141-51. Journal code: 9209120. ISSN: 0962-8819.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940818
Last Updated on STN: 19940818
Entered Medline: 19940811

L6 ANSWER 8 OF 1525 MEDLINE on STN
TI Synthesis and secretion of human serum **albumin** by mammary gland explants of virgin and lactating transgenic mice.
AB Transgenic mice were produced, carrying hybrid genes comprised of the ovine **beta**-lactoglobulin (BLG) milk protein gene promoter and human serum **albumin** (**HSA**) coding sequences. In situ hybridization revealed high levels of BLG/**HSA** hybrid mRNA, confined to the epithelial cells of the lactating mammary gland with a several hundred fold lower concentration in virgin mammary glands. During the first 24 h in culture, exceptionally high levels of **HSA** were secreted from explants of virgin mice, independent of hormonal control. **HSA** secretion was reduced considerably during subsequent days in culture and became dependent on the presence of insulin, hydrocortisone and prolactin. This temporal and hormonal pattern of regulation of **HSA** was different than that found for the secretion of caseins. In contrast to the vast difference in the mRNA content, the amount of **HSA** secreted from explants derived from lactating mice during the first 24 h in culture was only 2- to 5-fold higher than that found with explants from virgin transgenic mice, suggesting post-transcriptional control of **HSA** synthesis. The high-level synthesis and secretion of **HSA** in mammary explants of lactating mice was also dependent on the presence of insulin, hydrocortisone and prolactin. This study confirms previous suggestion that mammary explants from virgin transgenics may serve as a powerful tool for screening the potential of transgenic animals to secrete foreign proteins in their milk.

ACCESSION NUMBER: 94061080 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8242096
TITLE: Synthesis and secretion of human serum **albumin** by mammary gland explants of virgin and lactating transgenic mice.
AUTHOR: Barash I; Faerman A; Baruch A; Nathan M; Hurwitz D R; Shani M
CORPORATE SOURCE: Institute of Animal Science, ARO, Volcani Center, Bet Dagan, Israel.
SOURCE: Transgenic research, (1993 Sep) 2 (5) 266-76.

Journal code: 9209120. ISSN: 0962-8819.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 19940201
Entered Medline: 19940104

L6 ANSWER 9 OF 1525 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Expression of human serum **albumin** in milk of transgenic mice using goat **beta**-casein/human serum **albumin** **fusion** gene.

AB The gene encoding human serum **albumin** (**HSA**) was cloned from human liver cDNA library by PCR. The **HSA** cDNA in size of 2,176 bp, including 1,830 bp of open reading frame, was cloned into the plasmid carried with the 5' flanking sequence of goat **beta** -cascin gene (4,044 to +2,025 bp) to get a tissue specific expression vector in mammary gland named pGB562/**HSA** (12.5 kb). A 9.6 kb DNA fragment in which the sequence is in order of goat **beta** -casein gene regulatory sequence, **HSA** cDNA and SV40 polyadenylation signals was isolated from the pGB562/**HSA** by SacI and DraIII cutting, and used to microinject into the pronuclei of mouse fertilized eggs to produce transgenic mice. Three transgenic mice (2 female and 1 male) were identified by PCR and dot Southern blot analysis. The copy numbers of integrated transgene were more than 10 copies in line 21 and 26 as well as over 50 copies in line 31 of transgenic mice. **HSA** protein collected from the milk of lactating transgenic mice was confirmed by immuno-detection of Western and slot blot. The concentrations of **HSA** in the milk were from 0.05 to 0.4 mg/ml. An obvious antigen and antibody conjugate could be observed in immunohistochemical stain of mammary gland tissue from lactating day 11 of **HSA** transgenic mice. The transmission of transgene and its expression was recognized according to the results of RT-PCR and sequences analyses of their progeny.

ACCESSION NUMBER: 2004:438870 BIOSIS
DOCUMENT NUMBER: PREV200400437694
TITLE: Expression of human serum **albumin** in milk of transgenic mice using goat **beta**-casein/human serum **albumin** **fusion** gene.
AUTHOR(S): Wu, H. T.; Chou, C. K.; Huang, M. C. [Reprint Author]
CORPORATE SOURCE: Dept Anim Sci, Natl Chung Hsing Univ, Taichung, 402, Taiwan mchuang@mail.nchu.edu.tw
SOURCE: Asian-Australasian Journal of Animal Sciences, (June 2004) Vol. 17, No. 6, pp. 743-749. print.
ISSN: 1011-2367 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Nov 2004
Last Updated on STN: 17 Nov 2004

L6 ANSWER 10 OF 1525 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI An IFN-**beta**-**albumin** **fusion** protein that displays improved pharmacokinetic and pharmacodynamic properties in nonhuman primates.

AB The long half-life and stability of human serum **albumin** (**HSA**) make it an attractive candidate for **fusion** to short-lived therapeutic proteins. AlbuferonTM (Human Genome Sciences (HGS), Inc., Rockville, MD) **beta** is a novel recombinant protein derived from a gene **fusion** of interferon-**beta** (IFN- **beta**) and **HSA**. In vitro, Albuferon **beta**

displays antiviral and antiproliferative activities and triggers the IFN-stimulated response element (ISRE) signal transduction pathway. Array analysis of 5694 independent genes in Daudi-treated cells revealed that Albuferon **beta** and IFN-**beta** induce the expression of an identical set of 30 genes, including 9 previously not identified. In rhesus monkeys administered a dose of 50 mug/kg intravenously (i.v.) or subcutaneously (s.c.) or 300 mug/kg s.c., Albuferon **beta** demonstrated favorable pharmacokinetic properties. Subcutaneous bioavailability was 87%, plasma clearance at 4.7-5.7 ml/h/kg was approximately 140-fold lower than that of IFN-**beta**, and the terminal half-life was 36-40 h compared with 8 h for IFN-**beta**. Importantly, Albuferon **beta** induced sustained increases in serum neopterin levels and 2',5' mRNA expression. At a molar dose equivalent to one-half the dose of IFN-**beta**, Albuferon **beta** elicited comparable neopterin responses and significantly higher 2',5'-OAS mRNA levels in rhesus monkeys. The enhanced in vivo pharmacologic properties of IFN-**beta** when fused to serum **albumin** suggest a clinical opportunity for improved IFN-**beta** therapy.

ACCESSION NUMBER: 2003:171273 BIOSIS
DOCUMENT NUMBER: PREV200300171273
TITLE: An IFN-**beta**-**albumin fusion** protein that displays improved pharmacokinetic and pharmacodynamic properties in nonhuman primates.
AUTHOR(S): Sung, Cynthia; Nardelli, Bernardetta; LaFleur, David W.; Blatter, Erich; Corcoran, Marta; Olsen, Henrik S.; Birse, Charles E.; Pickeral, Oxana K.; Zhang, Junli; Shah, Devanshi; Moody, Gordon; Gentz, Solange; Beebe, Lisa; Moore, Paul A. [Reprint Author]
CORPORATE SOURCE: Human Genome Sciences, Inc, 9410 Key West Avenue, Rockville, MD, 20850, USA
paul_moore@hgsi.com
SOURCE: Journal of Interferon and Cytokine Research, (January 2003) Vol. 23, No. 1, pp. 25-36. print.
ISSN: 1079-9907 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Apr 2003
Last Updated on STN: 2 Apr 2003

L6 ANSWER 11 OF 1525 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Expression of recombinant psoriasis-associated fatty acid binding protein in Escherichia coli: Gel electrophoretic characterization, analysis of binding properties and comparison with human serum **albumin**.

AB The psoriasis-associated fatty acid binding protein (PA-FABP, also known as FABP5) is a novel keratinocyte protein that is highly up-regulated in psoriatic plaques (P. Madsen, H. H. Rasmussen, H. Leffers, B. Honore and J. E. Celis, J. Invest. Dermatol. 1992, 99, 299-305). Here we have expressed PA-FABP in Escherichia coli as a **fusion** protein containing an NH2-terminal hexa-His tag followed by a factor Xa cleavage site. The recombinant protein was expressed at a level of about 30% of the soluble proteins and was purified to homogeneity using a simple two-step protocol consisting of affinity chromatography on Ni2+-nitrilotriacetic acid agarose followed by gel filtration. The recombinant protein was then digested with factor Xa and characterized by two-dimensional gel electrophoresis. The ability of PA-FABP to bind saturated fatty acids ranging from 6 to 16 carbons was determined directly by dialysis and compared to human serum **albumin** (**HSA**). The results showed that PA-FABP binds multiple molecules of the fatty acids hexanoate (C6:0); octanoate (C8:0), decanoate (C10:0) and laurate (C12:0), all with a K1 of about 104 M-1, and myristate (C14:0) with a K1 of 4.4 X 105 M-1. Palmitate (C16:0) also bound strongly with multiple molecules. Due to the very low solubility of palmitate its affinity to PA-FABP was measured relatively to **HSA** and found to be 8.1 times

lower. At ligand/protein ratios below 1, all fatty acids bound to PA-FABP with about one to three orders of magnitude lower affinity than to **HSA**. The difference in the fatty acid binding properties of the two proteins may reflect differences in their three-dimensional structures, which in the case of PA-FABP consists mainly of **beta** -sheets while **HSA** contains predominantly alpha-helices.

ACCESSION NUMBER: 1998:405464 BIOSIS
DOCUMENT NUMBER: PREV199800405464
TITLE: Expression of recombinant psoriasis-associated fatty acid binding protein in Escherichia coli: Gel electrophoretic characterization, analysis of binding properties and comparison with human serum **albumin**.
AUTHOR(S): Vorum, Henrik; Madsen, Peder; Svendsen, Ib; Celis, Julio E.; Honore, Bent [Reprint author]
CORPORATE SOURCE: Dep. Med. Biochem., Univ. Aarhus, Ole Worms Alle, Build. 170, DK-8000 Aarhus C, Denmark
SOURCE: Electrophoresis, (July, 1998) Vol. 19, No. 10, pp. 1793-1802. print.
CODEN: ELCTDN. ISSN: 0173-0835.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Sep 1998
Last Updated on STN: 21 Sep 1998

L6 ANSWER 12 OF 1525 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Inhibition of influenza virus **fusion** by polyanionic proteins.
AB Anionic charge-modified human serum **albumin** (**HSA**) has previously been shown to exert potent in vitro activity against human immunodeficiency virus type 1 (HIV-1). In these studies, introduction of the additional negative charges was performed by derivatizing the epsilon-amino groups of lysine residues with succinic (Suc-**HSA**) or cis-aconitic anhydride (Aco-**HSA**), by which primary amino groups are replaced with carboxylic acids. The anti-HIV-1 activity was related to inhibition of gp41-mediated membrane **fusion**. Here, we investigated the activity of aconitylated and succinylated proteins on influenza virus membrane **fusion**, which is mediated by the viral membrane glycoprotein hemagglutinin (HA). Aco-**HSA** and Suc-**HSA** markedly inhibited the rates and extents of **fusion** of fluorescently labeled virosomes bearing influenza HA, with target membranes derived from erythrocytes. The inhibitory activity was dependent on the overall negative-charge density; **HSA** modified with 36 or less extra negative charges failed to inhibit **fusion**. The inhibition of **fusion** showed a certain degree of specificity for the protein carrying the negative charges: polyanionic **HSA** and **beta**-lactoglobulin A derivatives had **fusion** -inhibitory activity, whereas succinylated BSA, lactalbumin, lactoferrin, lysozyme, and transferrin were inactive, Aco-60-**HSA** and Aco-**beta**-lactoglobulin A inhibited influenza virus membrane **fusion** in a concentration-dependent manner, IC-50 values being about 4 and 10 mu-g/mL, respectively. HA-mediated membrane **fusion** is pH dependent. Aco-60-**HSA** did not induce a shift in the pH threshold or in the pH optimum. **Fusion** with liposomes of another low pH-dependent virus, Semliki Forest virus, was not specifically affected by any of the compounds reported here. In view of some structural and functional similarities between influenza HA and the HIV-1 gp120/gp41 complex, it is tempting to postulate that the current results might have some implications for the anti-HIV-1 mechanism of polyanionic proteins.

ACCESSION NUMBER: 1997:245963 BIOSIS
DOCUMENT NUMBER: PREV199799545166
TITLE: Inhibition of influenza virus **fusion** by polyanionic proteins.
AUTHOR(S): Schoen, Pieter [Reprint author]; Corver, Jeroen; Meijer,

Dirk K. F.; Wilschut, Jan; Swart, Pieter J.
CORPORATE SOURCE: Dep. Physiological Chem., Univ. Groningen, Ant. Deusinglaan 1, NL-9713 AV Groningen, Netherlands
SOURCE: Biochemical Pharmacology, (1997) Vol. 53, No. 7, pp. 995-1003.
CODEN: BCPCA6. ISSN: 0006-2952.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Jun 1997
Last Updated on STN: 9 Jul 1997

L6 ANSWER 13 OF 1525 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI **Beta**-lactoglobulin/human serum **albumin fusion** genes do not respond accurately to signals from the extracellular matrix in mammary epithelial cells from transgenic mice.
AB Mammary epithelial cell cultures from transgenic mice carrying the human serum **albumin** (**HSA**) gene or minigenes behind the regulatory sequences of the ovine **beta**-lactoglobulin gene were analyzed. Previously, we demonstrated that non-**HSA**-secreting transgenic strains retain the potential to express the **HSA** transgene in vitro and that mammary epithelial cell cultures from non-**HSA**-secreting strains express higher levels of **HSA** when grown on tissue culture plastic than they do when grown on collagen. In this study we studied the expression of BLG/**HSA fusion** genes in epithelial cell cultures of additional transgenic strains and additional substrata. Our results show that: (1) The BLG/**HSA fusion** gene in only one of seven **HSA**-secreting or nonsecreting transgenic strains tested accurately responded to signals from the EHS matrix; (2) **HSA** DNA sequences dominantly affected the activity of BLG as well as the whey acidic protein promoters; and (3) HGF/SF induced both milk proteins and **HSA** gene expression. These results suggest that the response to the extra cellular matrix (ECM) plays a key role in the expression of BLG/**HSA fusion** genes and that the function of the regulatory elements within the promoter regions of milk protein genes involved in response to the ECM, in developmental and in tissue specificity, very much depend on the downstream gene sequences.
ACCESSION NUMBER: 1996:535048 BIOSIS
DOCUMENT NUMBER: PREV199699257404
TITLE: **Beta**-lactoglobulin/human serum **albumin fusion** genes do not respond accurately to signals from the extracellular matrix in mammary epithelial cells from transgenic mice.
AUTHOR(S): Ilan, Neta; Barash, Itamar; Raikhinstein, Moshe; Faerman, Alexander; Shani, Moshe [Reprint author]
CORPORATE SOURCE: Inst. Animal Sci., Volcani Cent., Bet Dagan 50250, Israel
SOURCE: Experimental Cell Research, (1996) Vol. 228, No. 1, pp. 146-159.
CODEN: ECREAL. ISSN: 0014-4827.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Dec 1996
Last Updated on STN: 10 Dec 1996

L6 ANSWER 14 OF 1525 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Preparation and properties of recombinant G protein.
AB A gene for G protein from Streptococcus strain G148 was cloned in Escherichia coli, which gave rise to several plasmids. One plasmid containing a 1.5 kb insert coding for entire G protein with 63 kD. This protein had both an IgG binding capacity and **albumin**-binding activity. The second plasmid containing a 0.7 kD insert coded for protein with MM of 38 kD and had only an IgG-binding activity. The third coding

for protein with 25 kD has only **albumin**-binding activity. After subcloning the 1.5-kb insert into the other vector pSP65 and analysing the nucleotide sequence of this insert both in pSP65 vector, the authors came to the conclusion that the proteins obtained are **fusion** protein of G protein and **beta**-galactosidase. All the proteins were prepared by affinity chromatography on IgG sepharose or on **HSA** sepharose. The interaction between G protein and polyclonal and monoclonal IgG of the reactions between G protein and human IgG have determined.

ACCESSION NUMBER: 1996:267412 BIOSIS
DOCUMENT NUMBER: PREV199698823541
TITLE: Preparation and properties of recombinant G protein.
AUTHOR(S): Gupalova, T. V.; Golubkov, V. I.; Suvorov, A. N.; Andreev, A. S.; Dmitriev, A. V.; Totolyan, A. A.
CORPORATE SOURCE: Inst. Exp. Med., Russ. Acad. Med. Sci., St. Petersburg, Russia
SOURCE: Vestnik Rossiiskoi Akademii Meditsinskikh Nauk, (1996) Vol. 0, No. 3, pp. 44-50.
ISSN: 0869-6047.
DOCUMENT TYPE: Article
LANGUAGE: Russian
ENTRY DATE: Entered STN: 10 Jun 1996
Last Updated on STN: 10 Jun 1996

L6 ANSWER 15 OF 1525 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

TI Ectopic expression of **beta**-lactoglobulin/human serum **albumin** **fusion** genes in transgenic mice: Hormonal regulation and in situ localization.

AB We produced transgenic mice carrying the native sheep **beta** -lactoglobulin (BLG) or **fusion** genes composed of the BLG promoter and human serum **albumin** (**HSA**) minigenes. BLG was expressed exclusively in the mammary glands of the virgin and lactating transgenic mice evaluated. In contrast, transgenic females carrying the BLG/**HSA** **fusion** constructs also expressed the **HSA** RNA ectopically in skeletal muscle, kidney, brain, spleen, salivary gland and skin. Ectopic expression of **HSA** RNA was detected only in strains that express the transgene in the mammary gland. There was no obvious correlation between the level of the **HSA** RNA expressed in the mammary gland and that found ectopically. In three transgenic strains analysed, the expression of **HSA** RNA in kidney and skeletal muscle increased during pregnancy and lactation, whereas in the brain **HSA** expression decreased during lactation in one of the strains. **HSA** protein was synthesized in skeletal muscle and skin of strain 23 and its level was higher in lactating mice compared with virgin mice. Expression of **HSA** was also analysed in males and was found to be more stringently controlled than in females of the same strains. In situ hybridization analyses localized the expressed transgene in the skin, kidney, brain and salivary glands of various transgenic strains. Distinct strain-specific and cell-type specific **HSA** expression patterns were observed in the skin. This is in contrast to the exclusive expression of the **HSA** transgene in epithelial cells surrounding the alveoli of the mammary gland. Taken together, these results suggest that the absence of sufficient mammary-specific regulatory elements in the BLG promoter sequences and/or the juxtaposition of the BLG promoter with the **HSA** coding sequences leads to novel tissue- and cell-specific expression in ectopic tissues of transgenic mice.

ACCESSION NUMBER: 1994:398219 BIOSIS
DOCUMENT NUMBER: PREV199497411219
TITLE: Ectopic expression of **beta**-lactoglobulin/human serum **albumin** **fusion** genes in transgenic mice: Hormonal regulation and in situ localization.

AUTHOR(S): Barash, Itamar [Reprint author]; Faerman, Alexander; Ratovitsky, Tamar; Puzis, Raisa; Nathan, Margaret; Hurwitz, David R.; Shani, Moshe
CORPORATE SOURCE: Inst. Animal Science, ARO, Volcani Cent., Bet Dagan 50250, Israel
SOURCE: Transgenic Research, (1994) Vol. 3, No. 3, pp. 141-151. ISSN: 0962-8819.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Sep 1994
Last Updated on STN: 14 Sep 1994

L6 ANSWER 16 OF 1525 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
TI New isolated polynucleotide encoding a **fusion** protein formed between a human serum **albumin** (**HSA**) and a cell proliferation stimulatory factor (CPSF), useful for treating hematological disorders;
recombinant protein production via plasmid expression host cell for use in disease therapy
AN 2004-14446 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated polynucleotide encoding a **fusion** protein formed between a human serum **albumin** (**HSA**) and a cell proliferation stimulatory factor (CPSF), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a recombinant vector comprising the isolated polynucleotide; (2) a recombinant protein encoded by the polynucleotide; (3) a recombinant cell comprising the recombinant vector; (4) a composition comprising a combination of at least two different **HSA**/CPSF **fusion** proteins; (5) a method for treating a patient with a CPSF, or hematological disorder; and (6) a kit comprising a first **fusion** protein of **HSA** and a first CPSF, and a second **fusion** protein of **HSA** and a second CPSF.

BIOTECHNOLOGY - Preferred Molecule: The isolated polynucleotide encoding a **fusion** protein comprises a first nucleotide sequence at least 90 or 95% identical to a sequence of 1830 bp, fully defined in the specification, and a second nucleotide sequence encoding a CPSF positioned either 5'- or 3'- to the first nucleotide sequence, where the first and second nucleotide sequences are operably linked to be expressed as a **fusion** protein of **HSA** and CPSF. The first nucleotide encodes an amino acid sequence of 609 amino acids (P1), fully defined in the specification. The second nucleotide sequence is at least 90% identical to 600, 582, 630, 448 or 459 bp, fully defined in the specification. The second nucleotide encodes an amino acid sequence of 199, 193, 204, 152 or 144 amino acids, fully defined in the specification. The CPSF is selected from granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), (EOS)-CSF, CSF-1, EPO, interleukin-1 (IL-1); IL-2, IL-3, IL-4; IL-6; IL-7; IL-8, IL-9; IL-10; IL-11; IL-12; IL-13, IL-18, SLF, SCF, mast cell growth factor, EPA, Lactoferrin, H-sub-unit ferritin, prostaglandin (PG) E1 and E2, tumor necrosis factor-alpha (TNF-alpha), TNF-**beta**, TNF-alpha-1b, IFNalpha-2a, **interferon alpha**-2b (IFNalpha-2b), IFN-**beta**, IFN, ~~IFN-gamma~~; transfroming growth factor-**beta** (TGF-**beta**), activin, inhibin, leukemia inhibitory factor, oncostatin M, MIP-1alpha, MIP-1beta; MIP-2alpha, GRO-alpha; MIP-2-**beta**, platelet factor-4, macrophage chemotactic and activating factor and P-10. The isolated polynucleotide further comprises a third nucleotide sequence encoding a peptide linker that links the **HSA** and the CPSF. The peptide linker is a (G4S)3-4 linker. The length of the peptide linker is 2-100, 5-50, or 14-30 aa. The protein encoded by the polynucleotide binds to a specific antibody of human **albumin**. Preferred Vector: The vector is an expression vector for expressing the **fusion** protein in a host organism selected from mammal, fish, insect, plant, yeast, and bacterium.

The host organism is yeast selected from Saccharomyces, Candida, Pichia, Kluyveromyces, Torulaspora, or Schinosaccharomyces. The recombinant vector is a yeast transfer vector, such as pPICZ A, pPICZ B, or pPICZ C. Preferred Protein: The protein is recombinantly produced in yeast cells and glycosylated to substantially the same extent as that when recombinantly produced in mammalian cells. The mammalian cells are CHO cells. The yeast cells are Pichia pastoris cells. The protein has a shelf life at least 5 times longer than that of the CPSF alone when stored under the same condition. The protein has a plasma half-life at least 3 times longer than that of the CPSF alone when administered in vivo. Preferred Cell: The cell is selected from mammalian, fish, insect, plant, yeast, and bacterial cells. Preferred Composition: The composition comprises a combination of **HSA**/IL-11 **fusion** and **HSA**/EPO **fusion**; **HSA**/IL-3 **fusion** and **HSA**/EPO **fusion**; or **HSA**/IL-3 **fusion** and **HSA**/GCSF **fusion**. Preferred Method: Treating a patient with a CPSF comprises administering a pharmaceutical formulation comprising a **fusion** protein of **HSA** and CPSF to the patient. Treating a patient with a hematological disorder, comprises administering a first pharmaceutical formulation comprising a first **fusion** protein of **HSA** and a first CPSF to the patient in a therapeutically effective amount; and administering to the patient a second pharmaceutical formulation comprising a second **fusion** protein of HAS and a second CPSF to the patient in a therapeutically effective amount. This method can comprise administering the composition cited above. Preferred Kit: The kit comprises different first and second CPSF.

ACTIVITY - Hemostatic; Antianemic; Nephrotropic; Cytostatic; Anti-HIV; Immunosuppressive. No biological data given.

MECHANISM OF ACTION - Cell proliferation stimulatory factor.

USE - The polynucleotides, methods and compositions are useful for treating a patient needing CPSF or having hematological disorder (claimed), e.g. hypochromia, hypochromic microcytic anemia and anemia, platelet-less, HIV infection, cancer, renal failure and tissue/organ transplantation.

EXAMPLE - No relevant example given. (65 pages)

ACCESSION NUMBER: 2004-14446 BIOTECHDS
TITLE: New isolated polynucleotide encoding a **fusion** protein formed between a human serum **albumin** (**HSA**) and a cell proliferation stimulatory factor (CPSF), useful for treating hematological disorders; recombinant protein production via plasmid expression host cell for use in disease therapy
AUTHOR: YU Z; FU Y
PATENT ASSIGNEE: YU Z; FU Y
PATENT INFO: US 2004063635 1 Apr 2004
APPLICATION INFO: US 2003-609346 26 Jun 2003
PRIORITY INFO: US 2003-609346 26 Jun 2003; US 2002-392948 1 Jul 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-282521 [26]

L6 ANSWER 17 OF 1525 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
TI Secretory lysosome targeting **fusion** moiety for detecting and quantifying degranulation, containing protein or nucleotide encoding protein specifically localizes to secretory lysosome, label protein or nucleotide encoding label protein; involving vector plasmid pDsRED-mediated gene transfer and expression in host cell
AN 2004-09655 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A secretory lysosome targeting **fusion** moiety (FM) comprising a polypeptide or a nucleotide encoding the polypeptide that specifically localizes to a secretory lysosome and a label polypeptide or

nucleotide encoding the label polypeptide, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a cell (CL) comprising FM.

WIDER DISCLOSURE - Localizing secretory lysosomes in real-time within a cell using a targeted molecule, is also disclosed.

BIOTECHNOLOGY - Preferred **Fusion** Moiety: In FM, the polypeptide that specially localizes to a secretory lysosome comprises a protease chosen from tryptases, chymases and carboxypeptidases. The protease is chosen from mouse mast cell protease (MMCP) -1, -2, -3 -4, -5, -6, and -7, rat mast cell protease (RMCP) I and RMCP II, human chymases, human tryptases, cathepsin G-like protease, cathepsin G, carboxypeptidase A, and hexosaminidase, preferably rat mast cell protease (RMCP) II having accession number J02712. Preferred Cell: In CL, the label polypeptide is a fluorescent molecule, preferably Discosoma sp. red fluorescent protein or green fluorescent protein. The cell is chosen from mast cells, basophils, hemopoietic cells, melanocytes, and goblet cells, preferably mast cell. CL expressing FM is deposited at accession number PTA-45471.

USE - FM is useful in detecting and quantifying degranulation, which involves incubating CL in the presence of a cell activator, incubating the cell expressing the secretory lysosome targeting **fusion** moiety in the absence of the cell activator and detecting and quantifying the release of label in the supernatant in the presence of the cell activator compared to the release of label in the supernatant in the absence of the cell activator, where an increase in the release of label in the supernatant in the presence of the cell activator indicates degranulation. FM is useful in detecting and quantifying inhibition of degranulation, which involves incubating CL with a cell activator in the presence of a test substance, incubating the cell expressing the secretory lysosome targeting **fusion** moiety with the cell activator in the absence of the test substance and detecting and quantifying a change in the release of label in the supernatant in the presence of the test substance compared to the release of label in the supernatant in the absence of test substance, where a decrease in the release of label in the supernatant in the presence of test substance indicates inhibition of degranulation. FM is also useful in detecting and quantifying degranulation at the single cell level, which involves incubating CL in the absence of a cell activator, detecting and quantifying the amount of label in the absence of a cell activator, incubating CL in the presence of a cell activator, detecting and quantifying the amount of label in the presence of the cell activator, and detecting a change in the amount of label in the cell in the presence of the cell activator and detecting a change in the amount of label in the cell in the presence of the cell activator compared to the amount of label in the cell in the cell in the absence the cell activator, where a decrease in the amount of label indicates degranulation. The cell activator is chosen from IgE and a multivalent antigen, phorbol myristate acetate, ionomycin, compound 48/80, toll-like receptors, and protease receptors, preferably IgE and a multivalent antigen, phorbol, myristate acetate or ionomycin. FM is useful in increasing the purity of secretory lysosome preparation. FM is useful in studying secretory lysosome maturation, biosynthesis, cell differentiation, migration and activation in vivo. FM is also useful in delivering therapeutic polypeptides in vivo.

ADVANTAGE - FM efficiently detect and quantify degranulation at the single cell level and FM is effective in delivering therapeutic polypeptide in vivo.

EXAMPLE - Rat basophilic leukemia (RBL-2H3) cell line was used as a source of RNA. The sequence encoding rat mast cell protease (RMCP) II was cloned by PCR using the oligos such as 5'-tcagatctcgagatgcaggccctactattcc tg-3' and 5'-ctgcagaattcggctacttgtattaatgactgcat-3'. The PCR purified and digested with XhoI and EcoRI. The fragment was then cloned in the same sites of the pDsRED1-N1 (plasmid containing Discosoma sp. red fluorescent protein) vector and resulted in the full length RMCP II cDNA in-frame

with the N-terminus of the cDNA for the fluorescent protein DsRED. The sequence identity of the recombinant vector, RMCP-DsRED, was confirmed by DNA sequencing. RBL-2H3 cells (8x106) were transfected with the RMCP-DsRED vector (45 microg) by electroporation. The transfected cell line was designated RBL-RMCP/2C2. Transfected cells were transferred to the appropriate culture media and incubated for 48 hours. Positive clones were then selected by the addition of 1 mg/ml of active Geneticin. Ten days after transfection, the cells were analyzed by fluorescence activated cell sorting (FACS). A population of cells positive for red fluorescence was detected. Individual clones from the cell population were isolated by FACS and amplified. RBL-RMCP/2C2 was a positive clone expressing the RMCP-DsREd **fusion** protein. The subcellular localization of the RMCP-DsRED protein from the RBL-RMCP/2C2 clone was analyzed by confocal microscopy. A cellular clone expressing the pDsRED vector was used as control. The cells transfected with the pDsRED control vector expressed the DsRED protein in the cytoplasm. While RMCP-DsRED **fusion** showed punctuate expression in proximity to the plasma membrane and targeted to granules. On analysis, the RBL-RMCP/2C2 cells on stimulation with a mouse anti-DNA (dinitrophenyl) IgE followed by stimulation with DNA-**HSA** (DNP antigen coupled to human serum **albumin**) released histamine, **beta** hexosaminidase and the red fluorescence indicating that cells were degranulated. (29 pages)

ACCESSION NUMBER: 2004-09655 BIOTECHDS
TITLE: Secretory lysosome targeting **fusion** moiety for detecting and quantifying degranulation, containing protein or nucleotide encoding protein specifically localizes to secretory lysosome, label protein or nucleotide encoding label protein;
involving vector plasmid pDsRED-mediated gene transfer and expression in host cell
AUTHOR: RAJOTTE D; KABCENELL A
PATENT ASSIGNEE: BOEHRINGER INGELHEIM PHARM INC
PATENT INFO: WO 2004016212 26 Feb 2004
APPLICATION INFO: WO 2003-US25098 12 Aug 2003
PRIORITY INFO: US 2002-403464 14 Aug 2002; US 2002-403464 14 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-192061 [18]

L6 ANSWER 18 OF 1525 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
TI High quality reagent for testing male infertility, has glycosylated sperm binding glycopeptide complexed to detection agent, or zona pellucida protein capable of complexing with ZP3 or capable of binding to human sperm;
male infertility diagnosis system involving recombinant zona pellucida-2 protein
AN 2003-13627 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A high quality reagent (Ia) for testing male infertility, comprising properly glycosylated sperm binding glycopeptide (II) complexed to detection agent, a reagent (Ib) comprising recombinantly produced zona pellucida (ZP)-2 protein capable of forming ZP2/ZP3 complex upon incubation with ZP3, or a reagent (Ic) comprising a recombinant ZP2/ZP3 complex capable of binding specifically to human sperm, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a composition comprising purified human ZP2/ZP3 complex; (2) a kit for testing male infertility, comprising (Ia), (Ib) and (Ic) and at least a buffer solution or salt solution; (3) determining (M1) infertility of male by detecting one or more substances released from a sperm sample upon induction of the acrosome reaction in sperm of the sample; (4) an acrosome scoring method (M2) for determining infertility of a male, by inducing acrosome reaction in a sample of sperm from the male, detecting at least two different morphological types of acrosome reacted sperm, and calculating a score based on differential weighting of the detected at

least two different morphological types of acrosome reacted sperm; and (5) an artificial zona for detecting sperm binding, comprising a solid phase and at least one sperm binding agent comprising a minimum portion of rhZP3 that contains sperm binding site that binds sperm.

BIOTECHNOLOGY - Preferred Reagent: The glycopeptide in (Ia) is selected from properly glycosylated rhZP3, amino acid portion 310-345, 200-351 or 14-351 of rhZP3, at least one, two or three binding sites for human sperm, rhZP3, rhZP3/ZP2 complex, rhZP3/ZP2 **fusion** protein prepared as a single gene product, and rhZP3/ZP2/ZP1. The detection agent is selected from green fluorescent protein (GFP), a fluor, an enzyme, **beta**-galactosidase, alkaline phosphatase, horseradish peroxidase, an antigenic peptide, Alexa488, fluorophore-GFP conjugate, a peptide that comprises HA, V4 or Myc and biotin. The properly glycosylated sperm binding glycopeptide is prepared by culture of a uterogenital origin cell line or a mammary cell line, e.g. PA-1 or 293. In (Ic), the molar ratio of ZP3:ZP2 is at least1. The ZP2 is human ZP2. Preferred Method: One or more substances released from sperm sample in M1 are selected from enzyme, acid phosphatase, protein, membrane fragment, phospholipid, cholesterol, peptide, lipoprotein, nucleotide, nucleoside and glycoprotein. The substance is acrosin, where the method involves incubating the sperm with an acrosin inhibitor e.g. low pH solution, HCl, acetic acid, acidified amino acid and acidified glycine. In M2, at least three different sperm morphologies are detected and given weighting. Preferred Artificial Zona: The solid phase in the artificial zona is a particle or a substance selected from agarose, Sephadex, acrylamide, latex, polystyrene, glass, gold and insoluble protein. The solid phase is 25-250 microns in diameter. The sperm binding agent is selected from rhZP3, ZP2, ZP2-ZP3 complex, ZP1, ZP1-ZP3 complex, ZP1-ZP2-ZP3 complex, GFP-ZP3 and GFP-ZP3-ZP2. The sperm binding agent is attached to the solid phase in a manner that exposes a sperm binding portion of the sperm binding agent to the outside of the particle surface.

USE - (Ia), (Ib) or (Ic) is useful for detecting infertility of a male, by contacting (Ia), (Ib) or (Ic) with a sperm sample of the male. (Ia) is useful for detecting infertility of a male, by contacting (Ia) with a sperm sample of the male for a period of time sufficient to allow binding between the glycopeptide and the sperm and detecting the detection agent bound to sperm. The method further comprises washing the contacted sperm sample. The detection agent is an antigen, and the detection step involves incubating with a fluorescence labeled antibody to generate a fluorescence signal associated with bound detection agent. The fluorescence labeled antibody is Alexa 488 as well as other coupling dye coupled to an antibody that recognizes an antigenic site on the reagent e.g. ZP3/GFP (claimed). The reagents are useful for fertility testing.

ADVANTAGE - Fertility testing method using the above reagents are much easier to setup and monitor, allowing more convenient and inexpensive diagnostic testing for male fertility. The kids are useful for testing at a diagnostic laboratory or other facility. The methods are faster, convenient and enables rapid determination of male infertility.

EXAMPLE - Acrosin activity detection was carried out by measuring recombinant human zona pellucida protein (rhZP)-3. Semen was incubated at 37degreesC for 30 minutes to liquefy it. Basic parameters of the semen were then measured. The liquefied semen was added to the top of 90%/40% Percoll bilayers. The preparation was centrifuged for 20 minutes and the supernatant was discarded. The pellet was washed with HTF (human tubal fluid), which includes 0.5% human serum **albumin** (**HSA**). The material was then centrifuged for 10 minutes and the supernatant was discarded. Basic semen parameters were measured and the sperm concentration was adjusted to 20 million/ml. This was incubated for 5 hours at 37degreesC for capacitation, and the sperm was aliquoted to 500 mul each and Ca ionophore A23187 was added to a final concentration of 10 muM, 5 muM, 2.5 muM in separate reactions, which were incubated 1 hour at 37degreesC. The pellet was separated from the supernatant, washed and resuspended in 100 mul, ddH2O. The pellet/supernatant was adjusted to pH

4.0 with 0.1 M HCl, incubated 10 minutes and then substrate added in 1 ml buffer solution (pH 7.8). The optical densities of the samples were measured at 405 nM every 15 minutes for 2 hours. The data were fit to a liner regression model to extract the slope, Y=a+bX, where, Y is OD value and X is measured time intervals. The procedure was used to evaluate recombinant human ZP3. The recombinant human ZP-3 induced acrosome reaction at 30 ng/ml (i.e. minimally effective dose for sperm-zona pellucida binding inhibition in the hemizona assay) relative to control conditions (sperm culture medium or culture medium from non-transfected PA-1 cells: 19%+/-4.1% live acrosome-reacted sperm vs 9.2%+/-3.8% and 10.2%+/-2.7% live acrosome reacted sperm, respectively). (82 pages)

ACCESSION NUMBER: 2003-13627 BIOTECHDS
TITLE: High quality reagent for testing male infertility, has glycosylated sperm binding glycopeptide complexed to detection agent, or zona pellucida protein capable of complexing with ZP3 or capable of binding to human sperm; male infertility diagnosis system involving recombinant zona pellucida-2 protein
AUTHOR: CHI T; LIN Z; DONG K; HSU M; CHENG -; ZHENG H
PATENT ASSIGNEE: TRINITY BIOMEDICAL TECHNOLOGY CORP
PATENT INFO: WO 2003011118 13 Feb 2003
APPLICATION INFO: WO 2002-US24360 1 Aug 2002
PRIORITY INFO: US 2001-339632 11 Dec 2001; US 2001-309532 2 Aug 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-300454 [29]

L6 ANSWER 19 OF 1525 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
TI Novel human chemokine beta1 protein comprising deletion in amino acids from amino and/or carboxy terminus, and is a **fusion** protein further comprising human serum **albumin**, is useful for treating multiple sclerosis, asthma; vector-mediated recombinant protein gene transfer and expression in host cell for use in gene therapy
AN 2003-08682 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A human chemokine beta1 (Ckb1) protein (I) comprising a deletion in amino acid residues from amino terminus and/or carboxy terminus of a polypeptide having a 92 residue amino acid sequence (S1), given in the specification, is new.

WIDER DISCLOSURE - (1) full-length Ckb1 polypeptides, and its analogs or derivatives; (2) isolated nucleic acid molecules encoding (I), or the full-length Ckb1 polypeptides, and their antisense analogs; (3) antibodies against (I); (4) polynucleotides encoding Ckb1 polypeptide which is a **fusion** polypeptide further comprising the human serum **albumin** (**HSA**), expression vectors and host cells comprising the polynucleotides; (5) Ckb1 polypeptides or Ckb1 **fusion** proteins coupled to a detectable label; therapeutic or cytotoxic moiety; or a radioactive material; (6) antibodies that inhibit or abolish the binding of a CCR5 ligand, polynucleotides encoding the antibodies, methods of producing the antibodies, use of the antibodies in diagnostics or therapeutics, and use of the polynucleotides encoding the antibodies in gene therapy; (7) pharmaceutical preparations comprising the Ckb1 **fusion** proteins; (8) transgenic organisms modified to contain the above mentioned nucleic acid molecules; (9) polypeptides containing at least 80, preferably 99 % identity to a Ckb1 protein or Ckb1 **fusion** protein, and nucleic acids encoding these variants, fragments of the proteins; (10) polypeptides encoded by polynucleotides which hybridize to the complement of a nucleic acid molecule encoding the above mentioned amino acid sequences; (11) diagnostic kits comprising the antibodies; (12) primary, secondary, and immortalized host cells vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g. the coding sequence corresponding to a Ckb1 protein may be replaced with a

Ckb1-**HSA** coding region; (13) chemically modified derivatives of the Ckb1-**HSA fusion** proteins; (14) diagnostic assays involving the polynucleotides encoding the Ckb1 proteins, or the anti-Ckb1 antibodies; (15) gene therapy techniques involving the polynucleotides encoding Ckb1 protein; and (16) binding moieties that bind to Ckb1 protein identified by screening assays involving (I)-**HSA fusion** proteins.

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques. Preferred Protein: (I) is chosen from a polypeptide comprising residues 5-n, 6-n, 7-n, 8-n or 9-n, where n is any one of residues 56-74 of (S1). (I) further comprises first a heterologous protein such as human serum **albumin** (**HSA**). The **HSA** is at the N- or C-terminus of Ckb1. (I) further comprises a second heterologous protein at the N-terminus of Ckb1. The second heterologous protein is 4 amino acids in length and is selective for CCR5.

ACTIVITY - Anti-HIV; Neuroprotective; Antithyroid; Antiarthritic; Antirheumatic; Immunosuppressive; Nootropic; Antiinflammatory; Antiasthmatic; Antiallergic; Osteopathic; Nephrotrophic; Tuberculostatic; Virucide; Antiatherosclerotic; Antimicrobial.

MECHANISM OF ACTION - HIV replication inhibitor; CCR5 agonist or antagonist; Upregulates or downregulates CCR5 expression. The ability of Ckb1(G28-N93):human serum **albumin** (**HSA**) was determined as follows. Ckb1(G28-N93):**HSA** was solubilized in phosphate buffered saline (PBS) to a concentration of 4.4 mg/ml. Human immunodeficiency virus (HIV) strain Ba-L was obtained and grown exclusively in monocytes/macrophages. Peripheral blood monocytes were isolated from HIV-1 negative donors and then cultured for 6 days, allowing maturation of the cells to a macrophage-like phenotype. At day 6, the cultures were washed 3 times to remove any non-adherent cells and serially diluted test compounds were added. The compounds and cells were incubated at 37 degrees C for 60 minutes, and then a pre-titered amount of HIV-1 Ba-L virus added. The amount of virus to be used in the assays was determined by endpoint titration with and without azidothymidine (AZT). A volume of virus (titer) was selected which provides an inhibitory concentration of 50 % between 1 and 10 nm for AZT and greater than 500 pg/ml p24 by enzyme linked immunosorbent assay (ELISA) in virus control microtiter wells. Cultures were washed a final time by media removal 24 hours post-infection, fresh compound added and the culture continued for an additional 6 days. HIV p24 content was determined by ELISA to assess virus replication. cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction was performed on day 6 of the infection. AZT, HIV-1 reverse nucleoside transcriptase inhibitor was assayed in parallel as a positive control. Results showed that Ckb1(G28-N93):**HSA** inhibited HIV-1 replication with an IC50 of 1.6 mg/ml and no apparent cellular toxicity at 100 mg/ml. The positive control compound AZT provided an IC50 of 2.0 nM.

USE - (I) is useful for preventing infection, preferably viral (human immunodeficiency virus (HIV)) infection, in a cell, by contacting the cell with (I). (I) is also useful for treating a disease, such as HIV infection or immune disorders, hematopoietic disorders, autoimmune disorders, multiple sclerosis, Grave's disease, arthritis, rheumatoid arthritis, transplant rejection, neurodegenerative disorders, Alzheimer's disease, inflammatory disease, asthma, allergic disorders, inflammatory bowel disease, osteoarthritis, colitis, inflammatory kidney diseases, glomerulonephritis, infectious disease, tuberculosis, hepatitis infections, herpes viral infection, viral infection, proliferative disorders or atherosclerosis, in an individual (claimed). (I) inhibits or abolishes the ability of HIV to bind to, enter into/fuse with (infect), and/or replicate in CCR5 expressing cells. (I) also acts a CCR5 agonists or antagonists, stimulate chemotaxis of CCR5-expressing cells, inhibit CCR5 ligand binding to a CCR5 molecule, or upregulate or downregulate CCR5 expression. (I) is useful as an immunological probe for the differential identification of the tissues or cell-types. (I)-**HSA**

fusion proteins are useful for diagnosing, treating and preventing various disorders in mammals, preferably in humans. (I)-**HSA fusion** proteins are also useful as molecular weight markers on sodium dodecyl sulfate polyacrylamide gel electrophoresis techniques, for raising antibodies, and to test the biological activities of the Ckb1 protein. (I)-**HSA fusion** proteins are useful for screening for molecules that bind to the Ckb1 protein portion of the **fusion** protein. The **fusion** proteins are also useful in drug screening techniques.

ADMINISTRATION - (I)-human serum **albumin** (**HSA**) **fusion** protein is administered orally, parenterally, rectally, intracisternally, intravaginally, intraperitoneally, etc. Dosages of the **fusion** proteins administered parenterally range from 1 micro-g-10 mg/kg/day, most preferably for humans ranges from 0.01-1 mg/kg/day.

ADVANTAGE - The Ckb1 **fusion** proteins have increased stability, prolonged shelf-life and increased activity. The proteins exhibit selective binding to CCR5.

EXAMPLE - Vectors pScNHSA (ATCC Deposit Number PTA-3279) and pScCHSA (ATCC deposit Number PTA-3276) which are derivatives of pPPC0005 (ATCC Deposit Number PTA-3278) were used as cloning vectors into which polynucleotides encoding a chemokine beta1 (Ckb1) protein was inserted adjacent to and in translation frame with polynucleotides encoding human serum **albumin** (**HSA**). pScCHSA was used for generating Ckb1 protein-**HSA** fusions, while pScNHSA was used to generated **HSA**-Ckb1 protein fusions. Generation of pScCHSA was carried out as follows. The nucleic acid sequence encoding chimeric **HSA** signal peptide in pPc0005 was altered to include the XhoI and ClaI restriction sites. The XhoI and ClaI sites inherent to pPPC00005 (located 3' of the ADH1 terminator sequence) were eliminated. Then the XhoI and ClaI restriction sites were engineered into the nucleic acid sequence that encodes the signal peptide of HAS (a chimera of the **HSA** leader and a kex2 site from mating factor alpha, MAF) in pPPC0006 using two rounds of polymerase chain reaction (PCR). The resulting PCR product was then purified and digested with AflII and XbaI and ligated into the same sites in pPPC0006 creating pScCHSA. The presence of the XhoI site creates a single amino acid change in the end of the signal sequence from LDKR to LEKR. The D to E change will not be present in the final **albumin fusion** protein expression plasmid when a nucleic acid sequence comprising a polynucleotide encoding the Ckb1 portion of the **albumin fusion** protein with a 5' SalI site (which is compatible with the XhoI site) and a 3' ClaI site was ligated into the XhoI and ClaI sites of pScCHSA. Ligation of SalI to XhoI restores the original amino acid sequence of the signal peptide sequence. The pScCHSA was used as cloning vectors into which polynucleotides encoding a Ckb1 protein or fragment or variant was inserted adjacent to polynucleotides encoding mature **HSA**. pScCHSA was used for generating Ckb1-**HSA** fusions. DNA encoding a Ckb1 protein was PCR amplified. Once the PCR product was obtained it was cut with Bsu36I and one of (AscI, FseI, or PmeI) and ligated into pScNHSA. The presence of the XhoI site in the **HSA** chimeric leader sequence created a single amino acid change in the end of the chimeric signal sequence, i.e. the **HSA**-kex2 signal sequence, from LDKR to LEKR. An expression vector compatible with yeast expression was transformed into yeast Saccharomyces cerevisiae individual transformants were grown for 3 days at 30 degrees C in 10 mL YEPD (1 % w/v yeast extract, 2 % w/v, peptone, 2 % w/v, dextrose), and cells were collected at stationary phase after 60 hours of growth. supernatants were collected by clarifying cells. The protein expressed was isolated and then purified. (423 pages)

ACCESSION NUMBER: 2003-08682 BIOTECHDS

TITLE: Novel human chemokine beta1 protein comprising deletion in amino acids from amino and/or carboxy terminus, and is a **fusion** protein further comprising human serum **albumin**, is useful for treating multiple sclerosis, asthma;

vector-mediated recombinant protein gene transfer and expression in host cell for use in gene therapy
AUTHOR: BELL A; RUBEN S M
PATENT ASSIGNEE: HUMAN GENOME SCI INC
PATENT INFO: WO 2002097038 5 Dec 2002
APPLICATION INFO: WO 2002-US16525 24 May 2002
PRIORITY INFO: US 2001-293212 25 May 2001; US 2001-293212 25 May 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-140456 [13]

L6 ANSWER 20 OF 1525 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
TI **Beta**-lactoglobulin hybrid DNA constructs; human serum **albumin fusion** protein tissue-specific gene expression in transgenic animal milk
AN 1997-07466 BIOTECHDS
AB A new **beta**-lactoglobulin (BLG) hybrid construct contains BLG 5'-flanking sequences and BLG intragenic sequences in conjunction with a target cDNA, minigene or gene, preferably encoding human serum **albumin** (**HSA**). The intragenic sequences are BLG sequences downstream of the BLG translation initiation site (including sequences within BLG exon-1, intron-1 and/or exon-2), or BLG sequences within BLG exon-6, intron-6, exon-7, 3'-untranslated sequences and/or 3'-flanking sequences. The construct may be used to produce a transgenic mammal, to target expression of the product of the nucleic acid in the mammary gland of lactating female transgenic animals. Preferred mammals are mice, rabbits, sheep, goats, pigs or cattle, especially mice (e.g. a transgenic mouse produces **HSA** at 0.3 mg/ml of milk or higher). The nucleic acid is introduced into the mammal by e.g. microinjection of the BLG/DNA construct into an embryo of the mammal. The animals are tested for production of **HSA**, and those with highest production are bred. **HSA** can be isolated from the milk of these animals. (16pp)
ACCESSION NUMBER: 1997-07466 BIOTECHDS
TITLE: **Beta**-lactoglobulin hybrid DNA constructs; human serum **albumin fusion** protein tissue-specific gene expression in transgenic animal milk
AUTHOR: Barash I; Shani M; Nathan M; Hurwitz D R
PATENT ASSIGNEE: Min.Agr.Israel
LOCATION: Bet Dagan, Israel.
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